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# CALCIFEROLS AND BAIT SHYNESS IN THE LABORATORY RAT

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**ABSTRACT:** Rodenticides with delayed action are generally more effective than fast-acting compounds because of the phenomenon of bait shyness. Calciferols have a stop-feed effect quite soon after dosing, and physiological effects are measurable within one day of dosing. We investigated whether bait shyness might result from these fairly rapid effects in the laboratory rat. We found evidence of bait shyness following recovery from sub-lethal dosing with two forms of calciferol. Use of intubation as well as feeding showed that the response was to the bait carrier rather than to detection of calciferols *per se*.

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## INTRODUCTION

Calciferol is Vitamin D, a naturally occurring compound that is essential for healthy development in mammals. Vitamin D refers to a number of distinct but closely related compounds that were originally identified as possessing rickets-preventing (antirachitic) properties (Davidson and Wright 1972). Vitamin D<sub>2</sub> and Vitamin D<sub>3</sub> (Ergocalciferol and Cholecalciferol respectively) are the two 'activated' sterols of importance in nutrition and therapeutics. Ergocalciferol is manufactured by exposing ergosterol, a sterol found in fungi, to ultraviolet light, and is widely used in clinical practice. Cholecalciferol is the natural form of Vitamin D, and is produced by UV irradiation of 7-dehydrocholesterol, a sterol present in animal fats, including the oily secretions of mammalian skin and the preen gland of birds.

Vitamin D promotes the absorption of calcium and phosphate from the gut and is necessary for the formation of normal bone, although the underlying mechanisms are not clear. An overdose of Vitamin D promotes intestinal absorption of calcium and reabsorption of bone minerals, which can lead to hypercalcaemia, osteomalacia and metastatic calcification of the blood vessels (Meehan 1984); these symptoms are the basis of the rodenticidal properties of calciferols, and the immediate cause of death in small animals is often heart attack because of calcification of the blood vessels in the heart.

The acute oral LD<sub>50</sub> of calciferol for rats and mice is generally considered to be 30-100 mg•kg<sup>-1</sup> (Meehan 1984). Within the finished bait formulation, the concentration of calciferol is normally in the range 750-1000 mg•kg<sup>-1</sup>. However, palatabilities of formulations are variable, depending on the formulation base (Rowe et al. 1974). Following exposure to calciferol, food consumption is reduced over the following 1-3 days, presumably as a result of the animals suffering toxicosis, although there is no published evidence of the animals developing a subsequent bait shyness (Greaves et al. 1974, Zeinelabdin 1988). However, blood calcium levels are considerably elevated within 10-12 hours of ingestion of calciferol and it is possible that such a rapid physiological response might lead to bait shyness.

The laboratory study described here was initiated to investigate the reported 'stop feed' effect of calciferol on body weight and subsequent food consumption in laboratory rats. In addition, the animals that received a sub-lethal dose were allowed to recover, and then re-exposed to calciferol in a choice experiment. Comparison of their acceptance of calciferol bait with control animals that had no previous experience of calciferol would indicate whether the animals developed a form of 'bait shyness' or conditioned bait aversion (Prakash

1988) by association of the calciferol bait with the toxic effects. There were two sets of experiments. The first established the stop feed action of cholecalciferol bait and subsequent avoidance of the bait. The second used ergocalciferol administered by oral intubation as well as in food and demonstrated that the conditioned aversion or bait shyness was associated with memory of the bait carrier rather than with the previously mentioned rapid physiological response to the calciferol active ingredient (a.i.).

## CHOLECALCIFEROL: STOP-FEED ACTION AND BAIT SHYNESS

Cholecalciferol in the form of commercially prepared Quintox rodenticide was used in this initial investigation. Quintox is a pelleted formulation that contains 750 mg•kg<sup>-1</sup> of cholecalciferol, and is manufactured by Bell Laboratories Inc. (U.S.A.). Animals were initially exposed to a free choice of cholecalciferol pellets vs. ground food. After recovery from the toxic effects, survivors were offered a choice of either cholecalciferol pellets vs. ground food or another pelleted rodenticide vs. ground food.

### Materials and Methods

Groups of five male and five female Sprague Dawley rats, *Rattus norvegicus*, obtained from Charles River UK Ltd. were group-caged for a minimum of 7 days prior to the test, sexes separate, with food and water available ad lib. Animals were then caged singly for a conditioning period of two days prior to the test.

For the test, rats were exposed to cholecalciferol pellets for a period of one day, caged singly with a free choice of standard EPA Meal (Johnson and Prescott, in press) as an alternative food. The animals were allowed to recover for eighteen days, and were then either re-exposed to cholecalciferol pellets or exposed to Klerat pellets (an anticoagulant formulation containing 50 mg•kg<sup>-1</sup> brodifacoum) for a period of four days with a free choice of standard EPA Meal as an alternative food. Klerat pellets is of a similar formulation to cholecalciferol pellets, but without the calciferol active ingredient. From measurements of food consumption, the acceptance of the two rodenticides (defined as the weight of rodenticide consumed as a percentage of total food consumption) was compared with that of control groups of five male and five female animals not previously exposed to cholecalciferol.

Throughout the procedure, food consumption was measured daily and body weight was recorded every two to four days. During both conditioning and test periods, each cage had two food bowls held in place with metal clips to reduce

Table 1. Amount of cholecalciferol bait consumed and the calculated dose of cholecalciferol received by laboratory rats during the initial one day exposure. The animal codes identify individual females (F) and males (M).

(a) rats that survived the 18 day recovery period.

Animal	Bait consumed (g)	Active ingredient ingested (mg.kg <sup>-1</sup> )
F1	2.8	9.3
F2	2.4	9.1
M1	2.1	5.9
M2	3.7	10.5
M3	1.7	4.9
M4	4.2	12.6
F3	2.6	8.6
F4	2.6	8.8
M5	2.5	6.8
M6	1.5	4.5
M7	1.7	5.0
M8	1.4	4.2
Mean (± S.E.)	2.43 (± 0.25)	7.52 (± 0.78)

(b) rats that died during the 18 day recovery period.

Animal	Bait consumed (g)	Active ingredient ingested (mg.kg <sup>-1</sup> )
F5	2.1	7.7
F6	7.0	22.3
F7	3.1	10.6
M9	7.0	19.1
F8	2.5	8.2
F9	3.1	7.7
F10	3.7	12.6
M10	4.5	13.8
Mean (± S.E.)	4.12 (± 0.68)	12.75 (± 1.93)

spillage. Bowl position was reversed daily in case of positional bias. During the conditioning and recovery periods the animals were given a ground laboratory diet.

## Results

Total food consumption is shown in Fig. 1 for each of the surviving animals over the two days of conditioning, the test day (when exposed to cholecalciferol) and the first 14 days of the recovery period. The equivalent data for the control animals (that were not exposed to cholecalciferol) are presented in Fig.2. Changes in body weight over the same period for animals exposed to cholecalciferol are presented in Fig.3, and for the control animals in Fig.4.

Table 2. The effect of exposure to cholecalciferol pellets 18 days previously on acceptance of cholecalciferol pellets and of brodifacoum pellets in a four day choice test using laboratory rats and an alternative choice of EPA Meal.

Rodenticide	Initial exposure to cholecalciferol	Mean % acceptance (± S.E.)	
		First day of test	Four days of test
Quintox (cholecalciferol)	Yes	3.8 (± 1.4)	7.0 (± 1.6)
	No	21.7 (± 2.2)	18.7 (± 2.4)
Klerat (brodifacoum)	Yes	36.9 (± 6.4)	48.3 (± 3.9)
	No	30.6 (± 6.2)	43.7 (± 2.5)

The quantity of cholecalciferol bait consumed and the dose of active ingredient ingested during the initial 1 day exposure are shown in Table 1.

Following the initial exposure of cholecalciferol to the two groups of 10 rats, three females and one male from each group died (mean days to death 6.25; range 5-8). Animals that died were found to have consumed significantly more rodenticide than those that survived (two sample t test:  $t_9 = 2.7$ ,  $p < 0.05$ ). Of the surviving animals, over the first three days of recovery, daily food consumption of the eight males was reduced by 44.9% (SE = 9.5); and of the four females was reduced by 98.1% (SE = 0.3) (Fig. 1). Re-establishment of their previous daily consumption rates was achieved within 6 days in males, and within ten days in females, with the exception of one animal.

The surviving females consumed between 8.6 and 9.3 mg.kg<sup>-1</sup> of cholecalciferol, while the males consumed between 4.2 and 12.6 mg.kg<sup>-1</sup>. The reduction in daily food consumption during the three days following initial exposure to cholecalciferol was least pronounced in the males that ingested the least active ingredient, and most pronounced in the males that ingested the most active ingredient (Fig. 1; Table 1). Body weight was recorded on days 1, 2, 4, 7, 9, 11, 14 and 16. Over the first seven days of recovery, a reduction in body weight was observed in most animals that were exposed to cholecalciferol (Fig. 3). This reduction was more pronounced in females (mean reduction 14.7%;  $n = 4$ ; SE = 0.78) than in males (only five males had a reduced body weight; mean reduction 3.4%; SE = 0.9).

The acceptance of the two rodenticide formulations, determined using both animals that had received the 1 day exposure to cholecalciferol rodenticide eighteen days previously, and animals that had no previous experience of cholecalciferol rodenticide, are presented in Table 2.

The acceptance of cholecalciferol pellets over a four day choice test was reduced significantly in animals that had consumed a sub-lethal dose of cholecalciferol 18 days prior to test ( $t_{13} = 4.11$ ,  $p < 0.01$ ). Reduced acceptance was even more obvious after the first day of test ( $t_{13} = 6.85$ ,  $p < 0.0001$ ). The initial exposure to cholecalciferol had no effect on the acceptance of brodifacoum pellets (Table 2). The previous exposure to cholecalciferol had induced conditioned aversion to the cholecalciferol pellets.

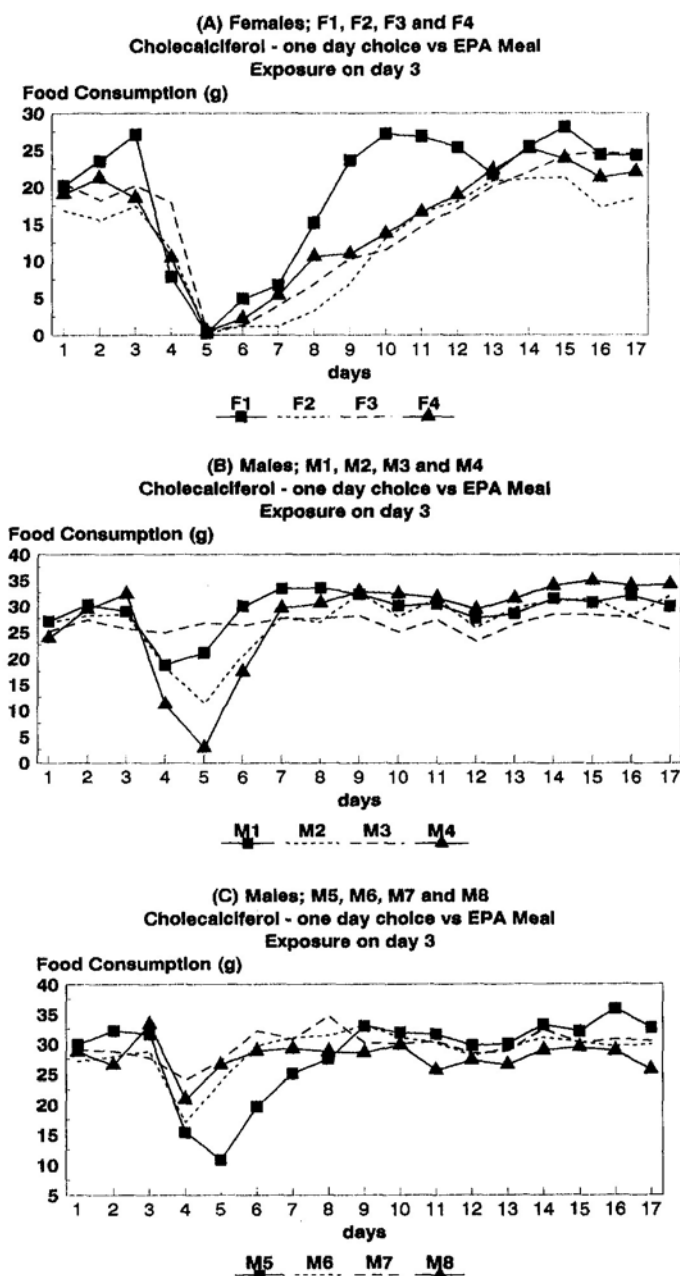


Figure 1. Total food consumption for each of the surviving animals, over the two days of conditioning, the test day (when exposed to cholecalciferol) and the first fourteen days of the recovery period. (A) = Females; F1, F2, F3 and F4; (B) = Males; M1, M2, M3 and M4; (C) = Males; M5, M6, M7 and M8.

### Summary

Cholecalciferol was more toxic to females than to males. After consumption of a lethal or a sub-lethal dose, the animals reduced or stopped feeding, and either recovered or died. Recovery was indicated by onset of feeding, eventually to normal consumption levels. The acceptance of cholecalciferol pellets was significantly reduced in animals exposed to cholecalciferol 18 days previously, providing evidence of conditioned bait aversion or bait shyness. Animals that recovered subsequently discriminated against cholecalciferol pellets.

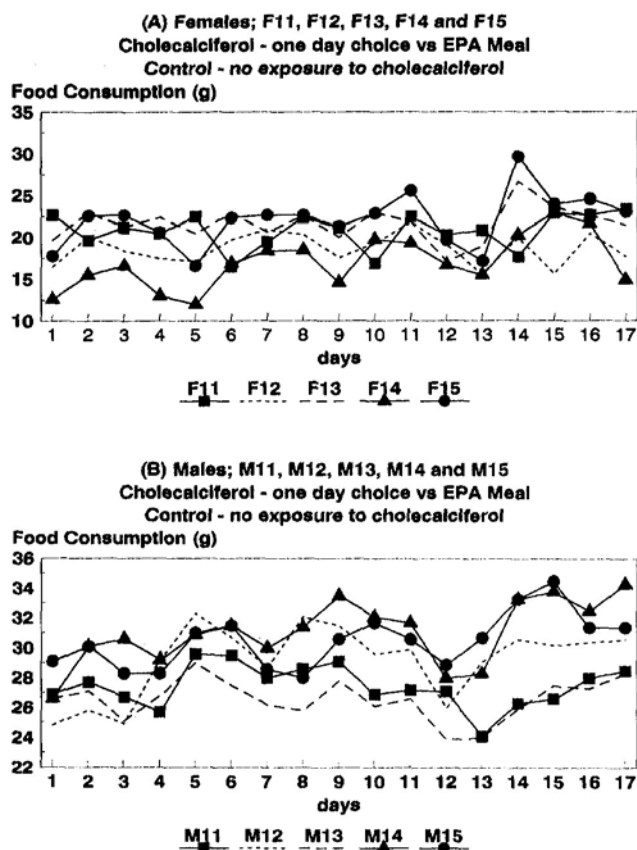


Figure 2. Total food consumption for the control animals over the two days of conditioning, the test day (with no exposure to cholecalciferol) and the first fourteen days of the recovery period. (A) = Females; F11, F12, F13, F14 and F15; (B) = Males; M11, M12, M13, M14 and M15.

### ERGOCALCIFEROL: IS BAIT SHYNESS A RESPONSE TO THE A.I. OR TO THE CARRIER BAIT?

Animals were exposed to ergocalciferol to provide comparative data to that for cholecalciferol regarding the stop feed effect and the development of bait shyness. Animals were presented with an initial exposure of ergocalciferol, either by oral intubation where the active ingredient dissolved in corn oil was delivered by gavage needle directly into the stomach, or by limited free feeding where animals of known weight were starved overnight and then fed a pre-determined quantity of bait containing  $750\text{mg}\cdot\text{kg}^{-1}$  ergocalciferol. In this way it was possible to deliver a predetermined quantity of active ingredient to each test animal.

Following an eighteen day recovery period, animals exposed to ergocalciferol were compared with unexposed control animals. Percent acceptance in a free choice test was determined for two non-toxic baits (Bait A- and Bait B-) and two baits containing ergocalciferol (Bait A+ and Bait B+). The four baits are one of two bait formulation bases, pinhead oatmeal (Bait A) or cornmeal (Bait B) and either contain ergocalciferol or not (+ or -). Bait A+ was that used to provide the initial exposure to ergocalciferol by limited free feeding.

The experiment was designed to answer the following questions:

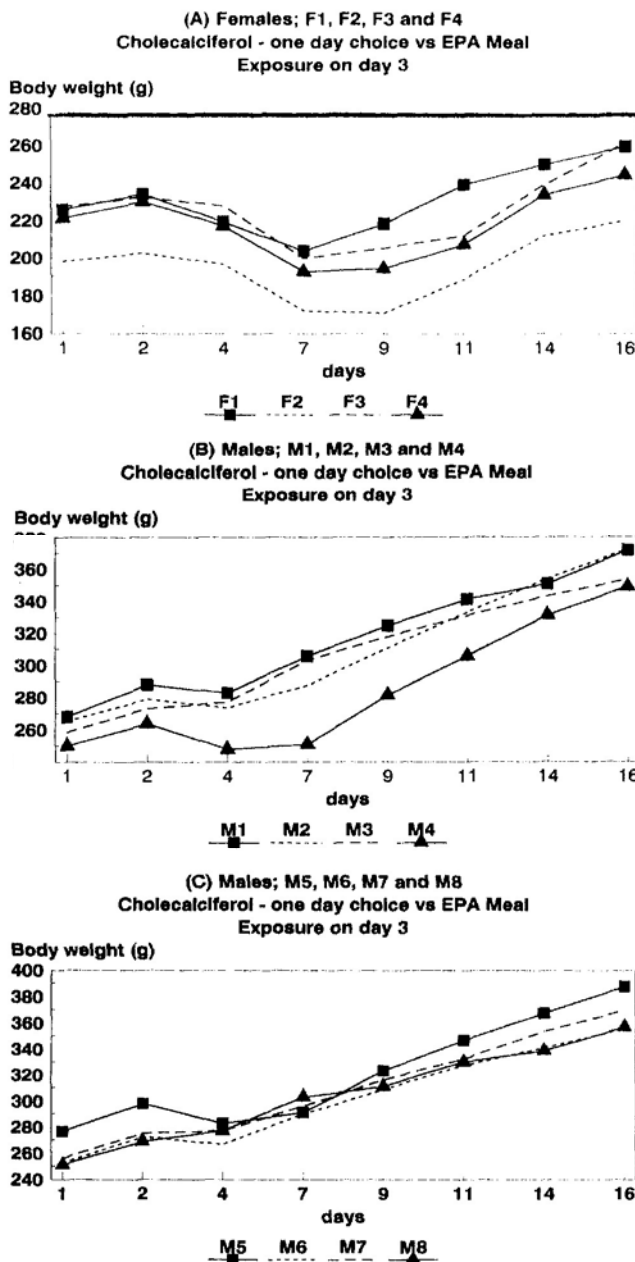


Figure 3. Changes in body weight for each of the surviving animals, over the two days of conditioning, the test day (when exposed to cholecalciferol) and the first fourteen days of the recovery period. (A) = Females; F1, F2, F3 and F4; (B) = Males; M1, M2, M3 and M4; (C) = Males; M5, M6, M7 and M8.

1. does ergocalciferol induce bait shyness?
2. does bait shyness result from memory of the taste of the bait carrier or the taste of the active ingredient, or is it a response to a rapid physiological effect of the active ingredient?

#### Materials and methods

The ergocalciferol was stored refrigerated and protected from light until required, when it was dissolved in corn oil immediately prior to use.

Four baits were prepared for this investigation.

Bait A- was prepared from pinhead oat meal (90% by

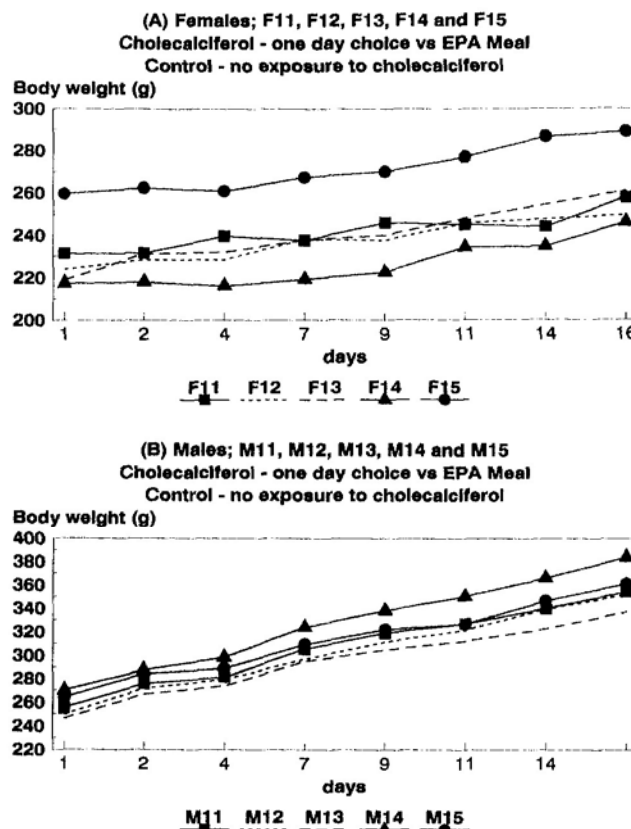


Figure 4. Changes in body weight for the control animals over the two days of conditioning, the test day (with no exposure to cholecalciferol) and the first fourteen days of the recovery period. (A) = Females; F11, F12, F13, F14 and F15; (B) = Males; M11, M12, M13, M14 and M15.

weight), confectioners sugar (5% by weight) and corn oil (5% by weight). Bait A+ was the same as Bait A-, but contained  $750\text{mg}\cdot\text{kg}^{-1}$  ergocalciferol (initially dissolved in the corn oil).

Bait B- was prepared from commel (90% by weight), confectioners sugar (5% by weight) and corn oil (5% by weight). Bait B+ was the same as Bait B-, but contained  $750\text{mg}\cdot\text{kg}^{-1}$  ergocalciferol (initially dissolved in the corn oil).

The experimental design was based on three initial treatments, an eighteen day recovery period, and four post-recovery tests (12 combinations) as shown in Table 3.

Twelve groups of five male and five female Sprague Dawley rats, *Rattus norvegicus*, were obtained from Charles River UK Ltd. and group caged for a minimum of 7 days prior to the test, sexes separate, with food and water available ad lib. After being housed individually in a test cage for a two day conditioning period, animals were starved overnight, weighed, and then fed the oatmeal bait with (A+) or without (A-) ergocalciferol at a rate of  $1\text{g}$  per  $100\text{g}$  body weight. Animals that received ergocalciferol by feeding (Bait A+) thereby received a dose of ergocalciferol of  $7.5\text{mg}\cdot\text{kg}^{-1}$  body weight. The animals normally consumed their limited feed within 1 hour. They were then re-weighed and intubated at a rate of  $0.5\text{ml}$  per  $100\text{g}$  body weight. Animals that received calciferol by intubation were intubated with corn oil containing the required amount of ergocalciferol to deliver

Table 3. The 12 combinations of initial treatments and post-recovery tests. Four baits were used in four day choice tests vs EPA Meal. Bait A was oatmeal, Bait B was cornmeal; + denotes bait with 750mg•kg<sup>-1</sup> ergocalciferol, - denotes bait without calciferol. The table shows the numbers of females (F) and males (M) used.

Initial treatment	Post-recovery test: choice vs EPA meal			
	Bait A+	Bait A-	Bait B+	Bait B-
Control (Bait A-)	5M + 5F	5M + 5F	5M + 5F	5M + 5F
Calciferol in food (Bait A+)	5M + 5F	5M + 5F	5M + 5F	5M + 5F
Calciferol by intubation (Bait A-)	5M + 5F	5M + 5F	5M + 5F	5M + 5F
Totals	15M + 15F	15M + 15F	15M + 15F	15M + 15F

7.5mg•kg<sup>-1</sup> body weight while other animals were intubated with corn oil only. Thus control animals and animals that received calciferol by intubation or by restricted free feeding were all subject to the same experimental procedures.

The animals were allowed to recover for eighteen days, and then groups of five male and five female rats from each of the three pre-treatments were presented with either Bait A+, Bait A-, Bait B+ or Bait B- for a period of three days given a free choice of either the test bait or standard EPA Meal as an alternative food. From measurements of food consumption, the acceptance of the four food types (measured as the amount of test bait consumed as a percent of total food consumption) was determined for each of the three pre-recovery treatments.

## Results

Total food consumption of the four food type (Bait A+, Bait A-, Bait B+ and Bait B-) over the post-recovery three day choice test, for each of the pre-recovery treatments (Control, Intubated calciferol and Fed calciferol) are presented for males and females separately in Table 4. The % acceptance of the four food types in the twelve combinations are presented for males and females separately in Table 5.

Animals that were presented an ergocalciferol bait as a free choice vs. EPA Meal were found to have consumed significantly less than animals presented a similar choice but without the ergocalciferol component.

Females that received an initial exposure to ergocalciferol by free feeding ingestion of Bait A+ were found to have a significantly lower acceptance of Bait A+ during the three day choice test vs EPA Meal than females that had no previous experience of ergocalciferol ( $t_5 = 2.69$ ;  $p = 0.05$ ).

Females that received an initial exposure to ergocalciferol by oral intubation did not have a significantly lower acceptance of Bait A+ than females that had no previous experience of ergocalciferol ( $t_7 = 0.77$ ;  $p = 0.47$ ).

Females that received an initial exposure to ergocalciferol by free feeding ingestion of Bait A+ did not have a significantly lower acceptance of Bait B+ during the three day choice test vs EPA Meal, than females that had no previous experience of ergocalciferol ( $t_7 = 0.10$ ;  $p = 0.92$ ).

Table 4. Mean food consumption (test bait + EPA alternative) for the four post-recovery choice-test baits. The initial treatments were either no exposure to ergocalciferol (Control), or exposure to ergocalciferol in food (A+), or exposure to ergocalciferol by intubation. Food consumption (g) is expressed as a mean of  $n = 5$  replicates ( $\pm$  S.E.).

## (a) Females

Initial treatment	Oatmeal		Cornmeal	
	A+	A-	B+	B-
No calciferol control	39.1 ( $\pm 4.1$ )	73.8 ( $\pm 5.2$ )	64.6 ( $\pm 9.5$ )	64.6 ( $\pm 4.4$ )
Fed calciferol (A+)	43.0 ( $\pm 3.0$ )	66.0 <sup>a</sup> ( $\pm 4.9$ )	57.7 ( $\pm 3.0$ )	87.7 ( $\pm 6.2$ )
Intubated calciferol	42.0 ( $\pm 2.4$ )	71.7 ( $\pm 5.5$ )	61.1 ( $\pm 5.5$ )	77.9 ( $\pm 8.2$ )

<sup>a</sup> $n = 4$  because one animal in this group died.

## (b) Males

Initial treatment	Oatmeal		Cornmeal	
	A+	A-	B+	B-
No calciferol control	53.1 ( $\pm 5.3$ )	86.2 ( $\pm 5.8$ )	79.6 ( $\pm 7.3$ )	105.4 ( $\pm 9.0$ )
Fed calciferol (A+)	52.9 ( $\pm 3.6$ )	86.5 ( $\pm 6.6$ )	72.2 ( $\pm 3.2$ )	101.9 ( $\pm 5.3$ )
Intubated calciferol	54.4 ( $\pm 3.8$ )	91.8 ( $\pm 4.5$ )	81.0 ( $\pm 7.1$ )	96.6 ( $\pm 4.3$ )

## (c) Statistical comparisons

Null hypothesis	Sex	t	d.f.	p
Equal consumption for A+ and B+	Females	8.56	21	$p < 0.0001$
	Males	8.95	25	$p < 0.0001$
Equal consumption for A- and B-	Females	4.04	27	$p < 0.0004$
	Males	4.76	27	$p < 0.0001$

Table 5. The effects of exposure to ergocalciferol 18 days previously on acceptance of two bait formulations (A oatmeal, B cornmeal) with (+) or without (-) ergocalciferol. The initial treatments were a control without calciferol, calciferol in food (A+) or calciferol by intubation. Data are presented as mean % acceptance ( $\pm$  S.E.)

(a) Females

Initial treatment	Post-recovery choice test			
	Oatmeal		Cornmeal	
	A+	A-	B+	B-
No calciferol control	77.5 ( $\pm$ 6.1)	64.1 ( $\pm$ 2.1)	15.7 ( $\pm$ 4.3)	19.6 ( $\pm$ 4.5)
Fed calciferol (A+)	59.5 ( $\pm$ 2.7)	66.4 ( $\pm$ 6.7)	16.2 ( $\pm$ 3.3)	14.0 ( $\pm$ 3.1)
Intubated calciferol	69.8 ( $\pm$ 7.9)	64.3 ( $\pm$ 7.3)	24.7 ( $\pm$ 3.2)	33.5 ( $\pm$ 7.8)

(b) Males

Initial treatment	Post-recovery choice test			
	Oatmeal		Cornmeal	
	A+	A-	B+	B-
No calciferol control	62.9 ( $\pm$ 4.8)	66.7 ( $\pm$ 5.4)	16.2 ( $\pm$ 3.4)	10.7 ( $\pm$ 2.8)
Fed calciferol (A+)	68.4 ( $\pm$ 7.6)	67.7 ( $\pm$ 8.8)	14.3 ( $\pm$ 4.5)	25.3 ( $\pm$ 5.6)
Intubated calciferol	69.7 ( $\pm$ 7.5)	88.2 ( $\pm$ 6.1)	15.0 ( $\pm$ 4.1)	27.2 ( $\pm$ 6.4)

Summary

Initial exposure to ergocalciferol in the form of Bait A+ induced bait shyness that was specific to Bait A+. Oral intubation of the active ingredient did not induce aversion to Bait A+. Initial exposure to ergocalciferol in the form of Bait A+ did not induce aversion to ergocalciferol in the form of Bait B+. Thus the oatmeal formulation of ergocalciferol induced conditioned bait aversion or shyness specific to that formulation. Consumption of ergocalciferol in a particular bait formulation (A+) induced symptoms of toxicosis which the animals subsequently associated with the formulation as a whole (A+) rather than to the taste of the ergocalciferol (present in B+) or the rapid physiological effects of calciferol (which would have occurred in animals intubated with calciferol and subsequently fed A- or B-).

DISCUSSION

Both cholecalciferol and ergocalciferol caused a reduction in overall food consumption (stop feed effect) and induced formulation specific bait shyness. In the field, where

there may be an alternative food supply at known feeding locations and where availability of new foods may be restricted by social behaviour, a proportion of animals would be expected to experience sub-lethal effects, thus inducing bait shyness specific to the calciferol formulation. Effective control could therefore become progressively more difficult to achieve. One theoretical solution could be to apply calciferol in a succession of different bait bases since the conditioned aversion reported here is a response to the formulation rather than to the active ingredient. However, this would not be a realistic practical solution in most cases.

Palatability of calciferol rodenticides are variable, depending on the base constituents of each formulation (Meehan 1984). However, there is some controversy over the development of bait shyness following the consumption of a sub-lethal dose of calciferol. Meehan (1984) makes the enigmatic statement when referring to calciferol, that "bait shyness does not appear to be a problem. Undoubtedly some experimenters and users would disagree with this."

Greaves et al. (1974) found no evidence of bait shyness with calciferol, as did Zeinelabdin (1988), where laboratory rats were given the free feeding choice of two foods (one containing calciferol). However, in a two-choice drinking test, Zeinelabdin (1988) demonstrated an aversion to sucrose solution that was sustained for 2 weeks, after an initial 2h exposure to the novel sucrose solution immediately followed by intubation with a sub-lethal dose of calciferol.

It may well be that bait shyness is an inconsistent problem with calciferols, depending on the bait formulation used and the alternative foods available. The latter point may be of particular relevance in the field. However, our results make clear that bait shyness to calciferol baits can develop, at least in laboratory rats.

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